

## The Theory of Mutagenesis

In this preliminary note we wish to express our doubts about the detailed theory of mutagenesis put forward by Freese (1959b), and to suggest an alternative.

Freese (1959b) has produced evidence that shows that for the  $r_{II}$  locus of phage T4 there are two mutually exclusive classes of mutation and we have confirmed and extended his work (Orgel & Brenner, in manuscript). The technique used is to start with a standard wild type and make a series of mutants from it with a particular mutagen. Each mutant is then tested with various mutagens to see which of them will back-mutate it to wild type.

It is found that the mutations fall into two classes. The first, which we shall call the base analogue class, is typically produced by 5-bromodeoxyuridine (BD) and the second, which we shall call the acridine class, is typically produced by proflavin (PF). In general a mutant made with BD can be reverted by BD, and a mutant made with PF can be reverted by PF. A few of the PF mutants do not appear to revert with either mutagen, but the strong result is that no mutant has been found which reverts identically with both classes of mutagens, and that (with a few possible exceptions) mutants produced by one class cannot be reverted by the other.

Freese also showed that 2-aminopurine falls into the base analogue class, and that most (85%) spontaneous mutants at the  $r_{II}$  locus were not of the base analogue type. We have confirmed this and shown that they are in fact revertible by acridines. We have also shown that a number of other acridines, and in particular 5-aminoacridine, act like proflavin (Orgel & Brenner, in manuscript).

Freese has produced an ingenious explanation of these results, which should be consulted in the original for fuller details. In brief he postulated that the base analogue class of mutagens act by altering an A—T base-pair on the DNA (A = adenine, T = thymine) into a G—C pair, or *vice versa* (G = guanine, C = cytosine, or, in the T even phages, hydroxymethylcytosine). The fact that BD, which replaces thymine, could act both ways (from A—T to G—C or from G—C to A—T) was accounted for (Freese, 1959a) by assuming that in the latter case there was an error in pairing of the BD (such that it accidentally paired with guanine) while *entering* the DNA, and in the former case after it was already in the DNA.

Such alterations only change a purine into another purine, or a pyrimidine into another pyrimidine. Freese (1959b) has called these "transitions." He suggested that other conceivable changes, which he called "transversions" (such as, for example, from A—T to C—G) which change a purine into a pyrimidine and *vice versa*, occurred during mutagenesis by proflavin. This would neatly account for the two mutually exclusive classes of mutagens, since it is easy to see that a transition cannot be reversed by a transversion, and *vice versa*.

We have been led to doubt this explanation for the following reasons.

Our suspicions were first aroused by the curious fact that a comparison between the *sites* of mutation for one set of mutants made with BD and another set made with PF (Brenner, Benzer & Barnett, 1958) showed there were no sites in the  $r_{II}$  gene, among the samples studied, common to both groups.

Now this result alone need not be incompatible with Freese's theory of mutagenesis, since we have no good explanation for "hot spots" and this confuses quantitative argument. However it led us to the following hypothesis:

*that acridines act as mutagens because they cause the insertion or the deletion of a base-pair.*

This idea springs rather naturally from the views of Lerman (1960) and Luzzati (in preparation) that acridines are bound to DNA by sliding *between* adjacent base-pairs, thus forcing them 6.8 Å apart, rather than 3.4 Å. If this occasionally happened between the bases on *one* chain of the DNA, but not the other, during replication, it might easily lead to the addition or subtraction of a base.

Such a possible mechanism leads to a prediction. We know practically nothing about coding (Crick, 1959) but on most theories (except overlapping codes which are discredited because of criticism by Brenner (1957)) the deletion or the addition of a base-pair is likely to cause not the substitution of just one amino acid for another, but a much more substantial alteration, such as a break in the polypeptide chain, a considerable alteration of the amino acid sequence, or the production of no protein at all.

Thus one would not be surprised to find on these ideas that mutants produced by acridines were not capable of producing a slightly modified protein, but usually produced either no protein at all or a grossly altered one.

Somewhat to our surprise we find we already have data from two separate genes supporting this hypothesis.

(1) The *o* locus of phage T4 (resistance to osmotic shock) is believed to control a protein of the finished phage, possibly the head protein, because it shows phenotypic mixing (Brenner, unpublished). Using various base analogues we have produced mutants of this gene, though these map at only a small number of sites. We have failed on several occasions to produce any *o* mutants with proflavin. On another occasion two mutants were produced; one never reverted to wild type, while the other corresponded in position and spontaneous reversion rate to a base analogue site. We suspect therefore that these two mutants were not really produced by proflavin, but were the rarer sort of spontaneous mutant (Brenner & Barnett, unpublished).

(2) We have also studied mutation at the *h* locus in T2L, which controls a protein of the finished phage concerned with attachment to the host (Streisinger & Franklin, 1956).

Of the six different spontaneous *h*<sup>+</sup> mutants tested, all were easily induced to revert to *h* with 5-bromouracil (BU)†. This is especially significant when it is recalled that 85% of the spontaneous *r*<sub>II</sub> mutants could not be reverted with base analogues (Freese, 1959b).

We have also shown (Brenner & Barnett, unpublished) that it is difficult to produce *h*<sup>+</sup> mutants from *h* by proflavin, though relatively easy with BU. The production of *r* mutants was used as a control.

It can be seen from Table 1 that if the production of *h*<sup>+</sup> mutants by BU and proflavin were similar to the production of *r* mutants we would expect to have obtained  $\frac{57 \times 26}{108} = 13h^+$  mutants with proflavin, whereas in fact we only found 1, and this may be spontaneous background.

† (Added in proof.) Five of these have now been tested and have been shown not to revert with proflavin.

Let us underline the difference between the *r* loci and the *o* and *h* loci. The former appear to produce proteins which are probably *not* part of the finished phage. For both the *o* and the *h* locus, however, the protein concerned forms part of the finished phage, which presumably would not be viable without it, so that a mutant can be picked up only if it forms an *altered* protein. A mutant which deleted the protein could not be studied.

TABLE I

	<i>r</i>	<i>h</i> <sup>+</sup>
BU	108	57
Proflavin	26	1

It is clear that further work must be done before our generalization—that acridine mutants usually give no protein, rather than a slightly modified one—can be accepted. But if it turns out to be true it would support our hypothesis of the mutagenic action of the acridines, and this may have serious consequences for the naïve theory of mutagenesis, for the following reason.

It has always been a theoretical possibility that the reversions to wild type were not true reversions but were due to the action of “suppressors” (within the gene), possibly very closely linked suppressors. The most telling evidence against this was the existence of the two mutually exclusive classes of mutagens, together with Freese’s explanation.

For clearly if the forward mutation could be made at one base-pair and the reverse one at a different base-pair, we should expect, on Freese’s hypothesis, exceptions to the rule about the two classes of mutagens. Since these were not found it was concluded that even close suppressors were very rare.

Unfortunately our new hypothesis for the action of acridines destroys this argument. Under this new theory an alteration of a base-pair at one place *could* be reversed by an alteration at a different base-pair, and indeed from what we know (or guess) of the structure of proteins and the dependence of structure on amino acid sequence, we should be surprised if this did not occur.

It is all too easy to conceive, for example, that at a certain point on the polypeptide chain at which there is a glutamic residue in the wild type, and at which the mutation substituted a proline, a further mutation might alter the proline to aspartic acid and that this might appear to restore the wild phenotype, at least as far as could be judged by the rather crude biological tests available. If several base-pairs are needed to code for one amino acid the reverse mutation might occur at a base-pair close to but not identical with the one originally changed.

On our hypothesis this could happen, and yet one would still obtain the two classes of mutagens. The one, typified by base analogues, would produce the substitution of one base for another, and the other, typically produced by acridines, would lead to the addition or subtraction of a base-pair. Consequently the mutants produced by one class could not be easily reversed by the mutagens of the other class.

Thus our new hypothesis reopens in an acute form the question: which back-mutations to wild type are truly to the original wild type, and which only appear to be

so? And on the answers to this question depend our interpretation of all experiments on back-mutation.

We suspect that this problem can most easily be approached by work on systems for which the amino acid sequence of the protein can be studied, such as the phage lysozyme of Dreyer, Anfinsen & Streisinger (personal communications) or the phosphatase from *E. coli* of Levinthal, Garen & Rothman (Garen, 1960). Meanwhile we are continuing our genetic studies to fill out and extend the preliminary results reported here.

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